

Treatment of Metastatic Renal Cell Cancer Using Multiple IV Administrations of IL-12 Plasmid Nanopolyplexes

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ABSTRACT

Renal cell cancer accounts for approximately 3% of adult malignancies and ~12,000 deaths per year. The most common site of metastatic involvement for this cancer is the lung where it appears in ~75% of the cases of metastatic disease. Treating primary lung tumors or tumors resulting from metastasis is exceptionally difficult as evidenced by poor patient outcome. While hormone therapy and chemotherapy are known to have little effect on metastatic renal cell carcinoma (RCC), high dose interleukin-2 and other immunotherapies have been shown to have some benefit. The strong anti-cancer properties of interleukin-12 (IL-12) make it a sought-after target for therapeutic development that could be particularly useful for treating RCC metastases. The effects of IL-12 are mediated through induction of local and systemic immune responses and strong anti-angiogenic characteristics and are associated with T-lymphocyte and natural killer (NK) cell proliferation, activation of cytotoxic T-lymphocytes and secretion of interferon-gamma (IFN- γ). The anti-angiogenic effects result from IFN- γ induced stimulation of MIG, IP-10 and direct inhibitory effects on VEGF mRNA.

For studies presented here, a novel polymeric delivery system (BD15K-12) has been developed. This PEI-based block co-polymer utilizes biodegradable linkages and is designed to produce high expression levels in vitro and in vivo. Results show that BD15K-12 formulated with a plasmid encoding for murine IL-12 and delivered intravenously (IV) is an effective inhibitor of experimentally induced metastatic lung tumors. For these studies Balb/c mice were injected (IV) with 1×10^5 Renca cells. At days 4, 9 and 16 after Renca cell administration mice were treated IV with formulated plasmid. The lungs were harvested 1 day after the last injection for cytokine analysis and tumor nodule quantification. Treatment produced significant levels of mL-12 in lungs that plateau at DNA dose levels above 9 μ g, while IFN- γ levels were increasingly higher up to a 30 μ g dose. A significant 85% dose-dependent reduction in the number of tumor nodules occurred in treated mice. Additional modifications were made to the polymer to reduce the moderate systemic toxicity associated with high treatment doses. Through a series of optimization studies it was shown that the addition of a specific amount of methoxy polyethylene glycol (mPEG) functional groups prevents an increase in liver enzymes following multiple IV administrations while preserving the overall efficacy level of treatment. The advantage of this approach is the ability to administer IL-12 repeatedly with minimal toxicity. Additional work is being performed to evaluate BD15-12 for use in administering inhibitory RNAs. Preliminary results have shown the ability to produce 70% knockdown of reporter gene following co-administration (IV) of formulated luciferase reporter gene plasmid with a plasmid encoding for a luciferase short-hairpin. Future experiments will be designed to examine combination approaches utilizing both therapeutic protein overexpression with RNAi for treating metastatic RCC.

INTRODUCTION

Interleukin-12 (IL-12) is known to have strong anticancer properties mediated through immunomodulation and inhibition of angiogenesis (Fig 1).

Systemic use of recombinant IL-12 has been associated with unacceptably high toxicities.

Use of polymers to deliver plasmid containing an IL-12 gene expression cassette may provide the ability to produce IL-12 for treating metastatic tumors in a safe and efficacious manner.

A novel biocompatible polymer has been developed and evaluated for use as a systemic delivery reagent of IL-12 gene and shRNA for treatment of metastatic renal cell carcinoma.

METHODS

The polymer BD15K-12 was generated from low molecular weight linear polyethylenimine (LPEI) that were produced using a uniform synthesis scheme to minimize molecular and structural heterogeneity. These polymers were then cross-linked via a biodegradable linkages to produce a multi-block copolymer of high transfection activity and low cytotoxicity. BD15K-12 polymer was further modified by attachment of activated mPEG to produce BD15K-12(PEG). Plasmid polymer complexes were prepared by mixing specified amount of a plasmid with polymer various nitrogen to phosphate (N/P) ratio in 10% sugar solution and incubating the mixture for 15 minutes at room temperature.

The murine and human IL-12 plasmid (p2CMVml-12) consists of the p35 and p40 gene each under control of a CMV promoter/enhancer and each containing SV40 late polyadenylation signal sequences. The shRNA plasmid constructs are from Open Biosystems, Inc (Huntsville, AL).

In vitro studies were performed using COS-1 cells. The cells were plated in 12-well plates at 150,000 cell/well in 10% FBS supplemented DMEM medium and grown to ~80% confluency.

For in vivo studies Balb/c mice were used. Plasmid was administered via tail vein injections. Various plasmid amounts were given in volumes of 300 μ l. To create the metastasis model, 1.0×10^5 RENCA (murine renal cell carcinoma) cells were administered IV in 200 μ l volume.

Cytokine measurements were by ELISA from R&D Systems (Minneapolis, MN)

RESULTS

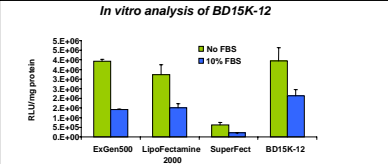


Fig. 2. Transfection efficiency of BD15K-12 in comparison to other commercially available reagents. The formulated plasmid encodes for the luciferase reporter protein. Experiments were performed in COS-1 cells with and without FBS. Values are means \pm SD.

In vivo metastatic tumor inhibition of interleukin-12 plasmid formulated with BD15K-12

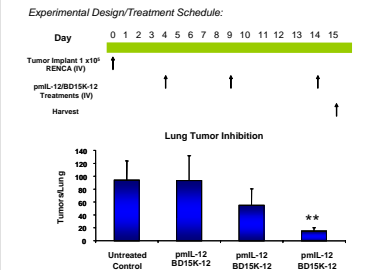


Fig. 3. Tumor bearing animals were treated three times with various doses of pml-12/BD15K-12. One day following that last treatment animals were sacrificed and tumor nodules in the lungs counted. Values are means \pm SD with a n=3 for each group. ** indicates significant difference from both the untreated control and 3 μ g dose groups.

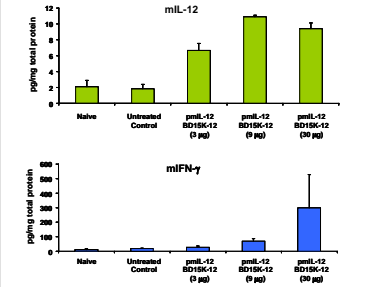


Fig. 4. Cytokine expression levels in lung of tumor bearing animals following IV administration of pml-12 formulated with BD15K-12. Lungs were harvested 24 hours after the last of three IV injections (15 days after tumor implant). Values are means \pm SD with a n=3 for each group.

PEGylation of BD15K-12 enhances in vitro transfection at high NP ratios

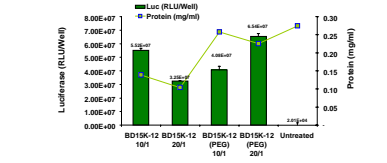


Fig. 5. Reporter gene expression levels in COS-1 cells following transfection with unmodified and PEGylated BD15K-12 at various NP ratios. PEGylation reduced cytotoxicity leading to higher overall expression levels at the 20/1 NP ratio. Values are means \pm SD.

In vivo comparison of anti-tumor activity: PEG modified vs. unmodified BD15K-12

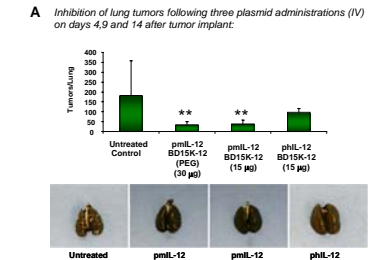


Fig. 7. Animals were treated with mIL-12 formulated with unmodified or PEGylated polymer. A plasmid encoding for human IL-12 was also used to evaluate non-specific anti-tumor activity. A. Tumor nodules found following treatment. Values are means \pm SD with a n=3 for each group. ** indicates significant difference from animals treated with formulated human IL-12 plasmid. B. Cytokine expression levels in lungs and serum following treatment. Values are means \pm SD with a n=4 for each group.

PEGylation of BD5K-12 reduces elevation of liver transaminases at high DNA concentrations

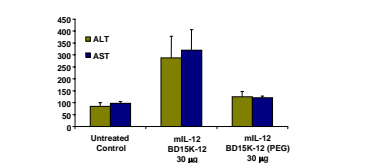


Fig. 6. Formulated plasmid (10/1, NP ratio) was administered (IV) into mice in a total volume of 300 μ l. Serum samples were collected for analysis 24 hours after treatment. Values are means \pm SD, n=6 per group.

Use of BD15K-12 polymer for in vivo RNAi delivery

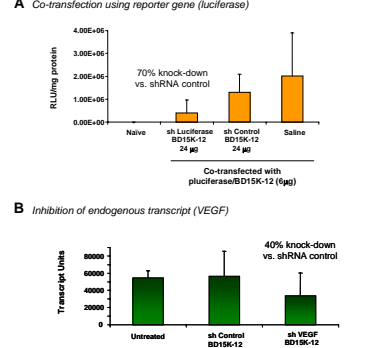


Fig. 8. Protein and transcript inhibition in mouse lung following shRNA delivery. A. Co-transfection experiment where plasmid encoding for luciferase and the sh constructs were administered IV simultaneously. Tissue was harvested for analysis 24 hours after treatment. All plasmid constructs were formulated with BD15K-12 (10/1 NP ratio). Values are mean \pm SD, n=3/group. B. VEGF transcript levels in mice following IV injection of formulated sh plasmid. qRT-PCR was performed using an ABI 7300 real-time PCR system. Values are mean normalized transcript units \pm SD, n=3/group.

CONCLUSIONS/SUMMARY

A non-viral plasmid delivery system (BD15K-12) has been developed that is a highly efficient in vitro transfection reagent.

Intravenous delivery of IL-12 gene formulated with BD15K-12 inhibits tumor formation in the lung in a murine model of metastatic renal cell carcinoma.

Modification/functionalization of BD15K-12 polymer by the covalent attachment of mPEG enhances transfection efficiency at high DNA concentrations in part by reducing toxicity. This translates into reduced elevation of liver transaminase levels following IV administration.

Use of BD15K-12 polymer and derivatives may be a useful reagent for IV delivery of gene based therapies including RNAi approaches.

Fig. 1. Multiple mechanisms of IL-12 action on tumor growth

